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Extrinsic regulators of epithelial tumor progression: metalloproteinases

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Extracellular metal-dependent proteinases regulate cell behavior by remodeling stromal and cell surface proteins, thereby influencing cell recruitment, cell shape, motility, proliferation, survival, genomic (in)stability, and differentiation. In recent years, the importance of proteinase-induced signaling has been underscored by evidence that altered regulation of cell–extracellular matrix and cell–cell interactions by proteinases can contribute, in a causal manner, to neoplastic progression.

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Abbreviations

ADAM	a disintegrin and metalloproteinase domain
DMBA	7,12-dimethylbenz[a]-anthracene
ECM	extracellular matrix
EGF	epidermal growth factor
FGF2	fibroblast growth factor-2
FGF-BP	fibroblast growth factor binding protein
HB-EGF	*heparin-binding epidermal growth factor-like growth factor*
IGF	insulin-like growth factor
IGFBP	insulin-like growth factor binding protein
MMP	matrix metalloproteinase
TGF	transforming growth factor
TIMP	tissue inhibitor of metalloproteinases
TNF-α	tumor necrosis factor-α
TPA	12-*O*-tetradecanoyl-phorbol-13-acetate

Introduction

The conversion of normal cells into invasive cancers with metastatic potential is a process that involves several steps. These steps are manifested in distinguishable histological and temporal stages — for instance, normal tissue, hyperplasia with a high incidence of proliferating cells, dysplasia with the induction of angiogenesis before the emergence of frank tumors with metastatic potential. Analysis of the later stages of tumor progression has resulted in a multi-step theory of tumorigenesis on the basis of genetic changes involving activation of oncogenes, inactivation of tumor suppressor genes, and altered expression of tumor-associated molecules. Whereas cancer research has historically focussed on such intrinsic events, it has become evident that extrinsic factors (e.g. the local stromal microenvironment) also regulate critical parameters of tumorigenesis and evolve and undergo multi-step reconstruction paralleling neoplastic progression. Thus, the stroma, and its resident reactive host cells, constitutes a second important component of solid tumors.

Requisite for neoplastic cell and capillary or inflammatory cell invasion during tumorigenic processes are the remodeling events that occur within the stroma or extracellular matrix (ECM). ECM-degrading proteinases are universally expressed during tumor progression and metastasis [1]. In epithelial tumors, the majority of proteinases are synthesized by the responding host stromal cells. The enzymes, if not membrane-spanning proteins, can then bind to the surface of tumor cells, to exert their biological activities. Extracellular proteinases are well known to degrade and/or remodel many substances in the ECM — such as cartilage, tendons, fibrin clots, basement membrane components — that allows for cell migration, deposition of new ECM, and the development of new tissue. Thus, extracellular proteinases are involved in essentially all developmental and pathological situations requiring tissue reorganization and remodeling. Although several types of extracellular proteinases are implicated in these events (metallo-, serine, and cysteine), members of the metzincin family (e.g. matrix metalloproteinases [MMPs]) and adamalysin-related proteinases, have emerged as important extrinsic regulators of tissue remodeling associated with neoplastic progression. Interestingly, new insights into *in vivo* proteinase substrates extend the role of metzincin proteinases beyond that of mere ECM dissolution [2,3•]. In this review we discuss recent insights into the regulatory functions of adamalysin-related and matrix metalloproteinases and their role in neoplasia, focussing on recently identified substrates as mediators of pericellular proteinase action. We first introduce the structural relationship between metzincin family members, then we highlight salient *in vivo* studies implicating certain metzincins as important extrinsic regulators of tumor growth.

The metzincin family

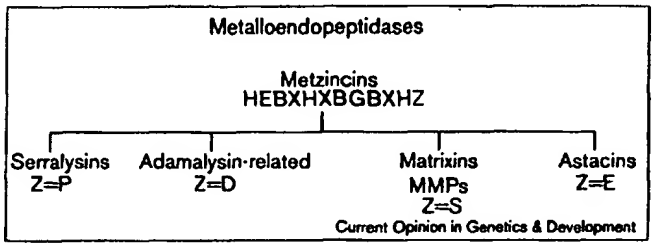
The metalloendopeptidases can be divided into several superfamilies and ~30 sub-families [4]. One superfamily, 'the metzincins' (Figure 1), are distinguished by a conserved structural topology, a consensus motif containing three histidines that bind zinc at the catalytic site, and a conserved 'Met-turn' motif that sits below the proteinase active site zinc ion (Figure 2) [5]. Thus, the metzincins can be further subdivided into four distinct families on the basis of structural similarity (Figure 3) and the identity of the ultimate Z residue (Figure 2) [5]. Two of these subfamilies are implicated in neoplastic events: the MMPs/matrixins and the adamylisin-related proteinases.

Matrix metalloproteinases

To date, 20 distinct vertebrate MMPs and 18 human homologues have been identified, as well as two non-vertebrate MMPs [1,2,3•]. The vertebrate MMPs each have distinct but often overlapping substrate specificities and

together they possess enzymatic activity against virtually all ECM components [1]. In addition to their dependence on zinc and calcium ions, the MMPs share several other common features. They show extensive sequence homology; they are all synthesized as inactive zymogens (or proMMPs) that are activated by limited proteolysis or exposure to organic mercurial compounds; they are either secreted or expressed as transmembrane proteins; they are universally inhibited by 1,10-phenanthroline, chelating agents and naturally occurring tissue inhibitors of metalloproteinases (TIMPs); they are transcriptionally responsive to cytokines, growth factors, hormones, and ECM-derived signals; and, as mentioned, they can hydrolyze ECM and non-ECM proteins [1]. Individual MMPs have been variously named, grouped and subdivided on the basis of their substrate specificities and either the presence or absence of specific functional protein domains (Figure 3).

Figure 1



Metzincin metalloendopeptidases. Major metzincin metalloproteinase families characterized by their signature three-histidine zinc-binding motif **HEBXHXBGBXHZ** [5], where H is histidine, E is glutamic acid, G is glycine, B is a bulky hydrophobic residue, X is a variable amino acid, and Z is a family-specific residue. The three H residues represent the catalytic triad. Thus, based on the 'Z' residue, four metzincin subfamilies have been described: Serralysins, adamylisin-related, Matrixins (MMPs), and Astacins.

Adamalysin-related proteinases

The adamalysins represent a family of soluble snake venom enzymes that aggressively degrade structural ECM components [5]. Together with snake venom disintegrins that contain the integrin-binding RGD sequence and prevent platelet aggregation, the adamalysins can cause rapid hemorrhage, tissue damage and necrosis. The family name, adamalysins, derives not from any domain considerations, but from the prototypic enzyme adamalysin II of the diamondback rattlesnake *Crotalus adamanteus*. The ADAMs, on the other hand, are cell-surface rather than secreted proteins that share *a* *d*isintegrin *a*nd *m*etalloproteinase domain and are thought to play important roles in fertilization, spermatogenesis, myogenesis, neurogenesis, somatogenesis, inflammation, and angiogenesis [6,7]. There are presently at least ~28 ADAMs that have been cloned from various species and tissue sources, each containing a signal sequence followed in order by a pro-domain, a metalloproteinase or metalloproteinase-like domain, a disintegrin-like domain, a cysteine-rich domain, EGF-like repeats, a transmembrane domain, and a cytoplasmic tail [6,7]. Accordingly, ADAMs potentially perform four distinct but complementary functions: proteolysis via the metalloproteinase domain, adhesion via the disintegrin domain, cell–cell fusion via a candidate hydrophobic fusion peptide in the cysteine-rich domain, and cell signaling via the intracellular domain which often contains sequences that

Figure 2

Structural model for the conserved zinc-binding region of metzincin proteinases. Invariant and distinguishing residues of the three-histidine zinc-binding motif and Met-turn. Within the signature three-histidine zinc-binding motif, H is histidine, E is glutamic acid, G is glycine, B is a bulky hydrophobic residue, X is a variable amino acid, and Z is a family-specific residue (i.e. aspartic acid [D] in adamalysins, E in astacins, proline [P] in serralysins, and serine [S] or occasionally threonine or valine in the MMPs). Likewise, the Met-turn consensus sequence UBMOJ (where M is methionine, and U, O and J are conserved family-specific residues) also distinguishes the various metzincin families from one another. Specifically, the U residue is a disulfide-bonded cysteine (C) in adamalysins, S in astacins and serralysins, and alanine (A) in most but not all MMPs. The O amino acid is usually H in astacins, S in serralysins, and A or tyrosine (Y) in the MMPs, and the J residue is a Y in astacins and serralysins, and P in all MMPs other than human stromelysin-3. Finally, O and J are both variable residues in the ADAMs/adamalysins. (Modified from [4,5].)

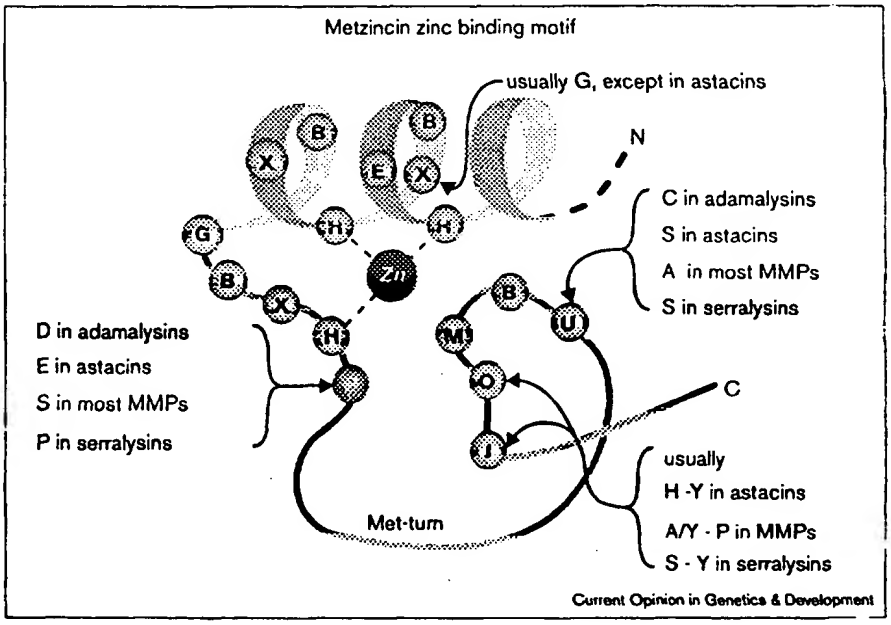
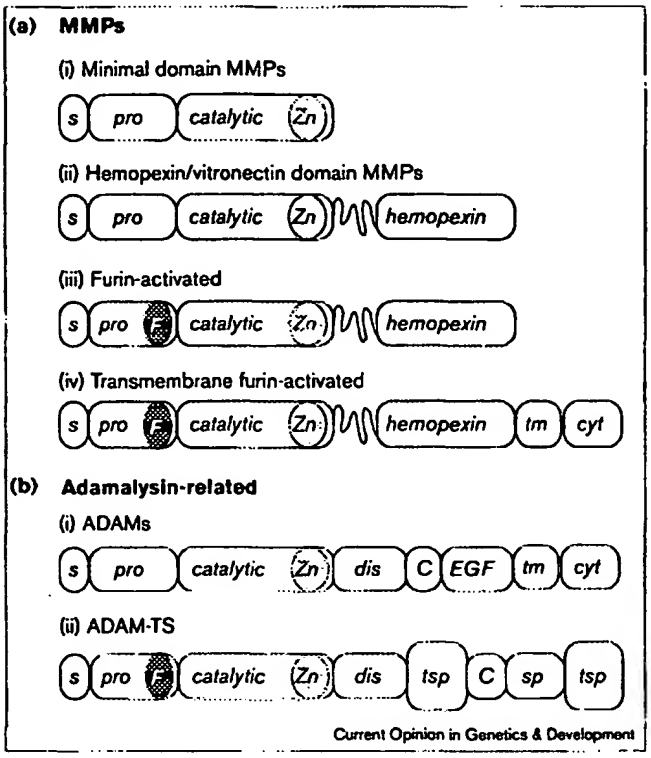


Figure 3



Domain structure of the (a) MMPs and (b) adamalysin-related proteinases. s, signal sequence; pro, propeptide with a free zinc-ligating thiol group; F, furin-like enzyme-recognition motif; Zn, zinc-binding site; dis, disintegrin-like domain; C, cysteine-rich domain; tsp, thrombospondin type I repeat; EGF, EGF-like domains; sp, spacer domain; *tm, transmembrane domain; cyt, cytoplasmic tail.* (*Modified* from [1,4,6].)

potentially bind SH3 domains of Src family members [7]. As the MMP family comprises soluble and membrane-bound proteinases, so does the ADAM family. Their soluble counterparts, ADAMTSs, do not contain a transmembrane domain but instead contain thrombospondin-1 motifs that permit ECM association [8••–10••]. It has been reported that there are currently 20 known human ADAMTS family members on the basis of homology and EST clone screening (S Apte, personal communication).

Modulation of tumor growth and expansion by metzincins

Proteolysis of ECM components serves several distinct functions — component assembly, editing of excess components and remodeling of ECM structure. These three processes are key to ECM synthesis and assembly, to physiological remodeling during growth, differentiation, morphogenesis and wound healing, and in pathological situations such as arthritis and tumor development. Several new, important insights into the mechanisms underlying these proteolytic processes as well as identification of new *in vivo* targets for proteinase action have been revealed that necessitate a fresh analysis of the role extracellular proteinases play during neoplastic progression — for example, the activation of growth factors, cleavage of cell–cell and cell–ECM adhesion proteins, release of bioactive ECM fragments, and induction of genomic instability (Figure 4).

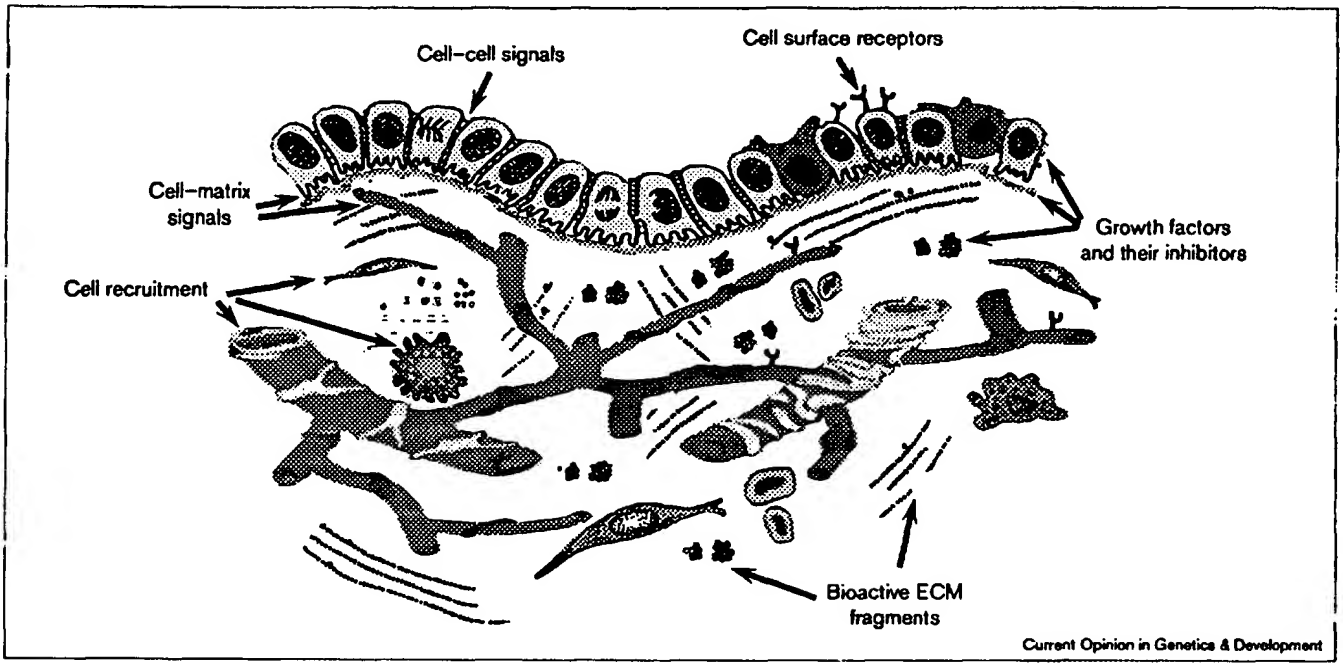
Liberation of sequestered growth factors

The balance between tumor cell proliferation and tumor cell apoptosis is a critical determinant of malignant cell outgrowth. Many autocrine and paracrine polypeptide growth factors have been identified that exert either direct mitogenic activity or cell survival capability, or both, on diverse tumor cell types. Regulation of polypeptide growth factor activity is complex. Some are secreted and found within the stromal matrix bound to various binding proteins — insulin-like growth factors (IGF-I and IGF-II), transforming growth factor-β (TGF-β), fibroblast growth factor-2 (FGF2) — whereas others are synthesized as pre-pro proteins — epidermal growth factor (EGF), TGF-α, heparin-binding EGF-like growth factor (HB-EGF), tumor necrosis factor-α (TNF-α), the c-kit ligand, colony stimulating factor 1 — and directed to the plasma membrane where, as latent precursors, they are proteolytically cleaved prior to the release of soluble activity. There has been great interest in identifying the mechanisms involved in mobilization of bound growth factors as well as identification of the enzymes responsible for proteolytic cleavage of cytokine and growth factor precursors, one reason being that release of these diverse factors contributes to pathological progression in multiple contexts.

IGFs have been implicated in both cell survival and the proliferation of diverse cell types [11,12••,13–16]. Their over-expression is frequently observed in hyperproliferative states that occur in human cancers of the prostate, breast, and liver [11,12••]. Control of IGF bioactivity is complex. Extracellularly, IGF activity is controlled by several high-affinity extracellular binding proteins (IGFBPs 1–7) [13]. The molar excess of IGFBPs, along with their high affinity for IGFs, leads to effective sequestration of IGFs, resulting in little free IGF in most contexts. Nonetheless, proteolytic cleavage of IGFBPs by several MMPs — stromelysin-3/MMP-11, interstitial collagenase-1/MMP-1, stromelysin-1/MMP-3, gelatinase A/MMP-2 and gelatinase B/MMP-9 — as well as some aspartic and serine proteinases, has now been demonstrated [14–16]. Furthermore, recent work has shown that IGF-II signaling in hepatic neoplastic progression is blocked by over-expression of the MMP inhibitor TIMP-1 [12••]. Thus, IGFBP proteolysis may represent a mechanism for tissue-specific regulation of IGF bioavailability, either enhancing and/or inhibiting IGF activity and further implicate the control of MMP-mediated degradation of IGFBPs as a novel therapy for controlling IGF bioavailability in cancer.

Another stromally sequestered polypeptide growth factor whose expression has been critically linked to tumorigenesis is basic FGF/FGF-2. FGF-2 is a potent growth factor and mitogen for many cell types. Notably, overexpression of FGF-2 and its receptor have been observed in many

Figure 4



Expanded role for pericellular proteinases. Metalloendoproteinases of the MMP and adamalysin-related families regulate cellular proliferation, cell recruitment, angiogenesis, migratory capability, and neoplasia-associated genomic instability. Recently identified *in vivo* targets facilitating these activities include cryptic bioactive fragments of ECM, sequestered growth factors and their inhibitors, latent cell surface ligands (polypeptide growth factors, chemokines and cytokines) and their receptors, and homophilic and heterophilic cell adhesion proteins.

human cancers, where it is believed to function mainly in tumor-associated angiogenesis [17]. Similar to IGFs, regulation of FGF-2 is complex. Following synthesis, FGF-2 is sequestered within the matrix (basement membranes primarily) by at least two different binding proteins that regulate FGF-2 bioavailability in a positive and negative manner, respectively. Perlecan, a major heparan sulfate proteoglycan of the pericellular environment binds FGF-2 with high affinity, sequestering latent reservoirs of the factor at the cell surface and within basement membranes. Perlecan is readily degraded by stromelysin-1/MMP-3, interstitial collagenase-1/MMP-1, plasmin, and several heparanases, that thus modulate bioavailability of FGF-2. [18]. Another binding protein for FGF-2 is FGF-BP. Interestingly, interaction of FGF-2 and FGF-BP does not limit bioavailability of the growth factor similar to FGF-2/perlecan or IGF/IGF-BP interactions, instead, FGF-BP mobilizes and activates locally stored FGFs [19]. In this case (as of yet unidentified) proteinases that target FGF-BP would negatively regulate FGF-2 bioavailability. Thus, the ECM is an abundant source of FGF-2 that can be targeted by proteinases to either sequester and limit mobilization of the growth factor or facilitate rapid and focal angiogenic responses that precede transcriptional induction of the factor in early neoplastic cells.

Shedding of membrane-bound latent growth factors

The extracellular domains of many transmembrane proteins can be proteolytically cleaved (shed) and released into the pericellular environment [2]. Ectodomain shedding can convert membrane-anchored growth factors/ cytokines into diffusable factors as well as rendering cell adhesion molecules incapable of either homophilic or heterophilic interactions (see below). There has been great interest in identifying the enzymes responsible for these proteolytic cleavage events as conversion of insoluble precursors to soluble factors by enzymatic cleavage constitutes an important post-translational modification that regulates physical location, activity, and bioavailability. Furthermore, that diverse cell surface protein ectodomains are shed by mechanisms sensitive to MMP inhibitors suggests a pericellular therapeutic opportunity by targeting identified proteinases [20]. During tumorigenesis, many of these proteins are of practical importance, as changes in their spatial and temporal expression characteristics parallel neoplastic progression.

TNF-α is a potent mediator of inflammatory reactions [21]; inflammation is, in turn, a potent mediator of tumor-associated angiogenesis [22••]. This cytokine is synthesized as a transmembrane precursor that is processed by proteolysis to a soluble homotrimer by a proteinase (TACE or ADAM17) that is a member of the ADAMs/adamalysin family [23,24]. Interestingly, analysis of mice lacking ADAM17 revealed an expanded role for the enzyme in inducing ectodomain shedding of other cell-surface proteins, including a TNF receptor, L-selectin, and the oncogenic growth factor TGF-α [25••], and broadly implicating ADAM17 in many aspects of neoplastic growth. Furthermore, a compelling role for

TNF-α in tumor progression has been revealed by examining skin carcinogenesis in TNF-α-deficient mice [26••]. TNF-α-deficient mice are resistant to benign and malignant skin tumor development, whether induced by initiation with DMBA and promotion with TPA, or by repeated dosing with DMBA. Thus, strategies that neutralize TNF-α production, or bioavailability, may be useful in cancer treatment and prevention.

Similar to TNF-α, members of the EGF family — EGF, TGF-α, HB-EGF, amphiregulin, the neu differentiation factors, and betacellulin — are synthesized in latent form as transmembrane precursors [20,27]. These polypeptide growth factors possess mitogenic and/or chemotactic activities toward a wide variety of cell types, altered expression for several has been reported in a wide variety of human cancers, and ectopic over-expression of some in transgenic mice reveals discrete oncogenic properties resulting in induction of neoplastic growth. Whereas ADAM17 releases soluble TGF-α, stromelysin1/MMP-3 releases HB-EGF [28]. Both ADAM17 and MMP-3-mediated shedding is inhibitable by TIMPs [20,29,30] and by synthetic MMP inhibitors [31]. Although the physiological significance of ectodomain shedding remains in most cases unknown, the existence of a common system for membrane protein ectodomain shedding involving metzincins is clear. Moreover, that surface localization of these enzymes essentially restricts their activity to specific microenvironments and release of their targets leads to both autocrine and distal paracrine effects motivates intense examination of *in vivo* substrates directly regulating cellular physiology.

Cell adhesion molecules as proteinase substrates

Development of malignant tumors, specifically the conversion of a benign lesion into an invasive cancer, is critically linked to the neoplastic cell's ability to overcome cell–cell and cell–ECM adhesive constraints, thereby acquiring invasive capability. Consequently, certain adhesion molecules have now been identified as contributors to the malignant phenotype [32••], some of which have recently been identified as substrates for metzincin family members.

E-cadherin-mediated adhesion (which is homophilic) is lost during the development of most, if not all, human epithelial cancers, including breast, colon, prostate, stomach, liver, esophagus, skin, kidney, and lung, where deletion or mutational inactivation of the gene has been reported in many cases [32••]. Furthermore, reduced and/or loss of E-cadherin expression has now been causally demonstrated to result in reduced malignant potential in cancer-prone transgenic mice [32••,33••] — thus, E-cadherin is considered a tumor suppressor protein [32••]. Stromelysin-1/MMP-3 cleaves the extracellular domain of E-cadherin expressed in mammary epithelial cells, triggering progressive phenotypic conversion characterized by disappearance of E-cadherin and catenins from cell–cell contacts, downregulation of cytokeratins, upregulation of vimentin, induction of keratinocyte growth factor expression and activation, and upregulation of endogenous MMPs [34,35]. Moreover, stromelysin-1/MMP-3 can trigger epithelial→mesenchymal phenotypic conversion of cultured mammary epithelial cells and induces otherwise non-invasive and non-tumorigenic cells to form highly infiltrative tumors in immunocompromised mice [34–36]. These data indicate that, in addition to its traditional pro-invasive activity [35,36], stromelysin-1/MMP-3 can initiate the development of early premalignant lesions in the breast and can foster late phenotypic changes associated with more aggressive tumor behavior, perhaps in part as a result of its interaction with E-cadherin.

Proteinases as oncogenes: stromelysin-1/MMP3 promotes carcinogenesis

Stromelysin-1/MMP-3 was originally cloned as a tumor-specific gene and has been shown to be upregulated in a variety of human and rodent cancers [1]. Stromelysin-1/MMP-3 is expressed in stromal cells throughout mammary development and is maximally expressed during involution when ECM remodeling and alveolar regression takes place. Interestingly, Werb and co-workers have demonstrated that ectopic expression of stromelysin-1/MMP-3 in glandular epithelium promotes malignant conversion inducing invasive mesenchymal-like tumors in the mammary glands of mice [35,37••], while also promoting stereotyped genomic changes unlike those observed in other murine mammary cancer models [37••]. Notably, continued expression of stromelysin-1/MMP-3 is not required for sustenance of either the tumor phenotype or induced genomic alterations, suggesting that stromelysin-1/MMP-3 truly can act by a 'hit-and-run' mechanism.

If proteinases can act as initiators of neoplasia in mice, then mutations, amplification, or polymorphism's in metzincins may be associated with tumor development in humans. Interestingly, a polymorphism in the human collagenase-1/MMP-1 gene promoter that creates a transcription-enhancing Ets site occurs more often in tumor cell lines than in the general population [38•]. This suggests that enhanced MMP-1 transcription may contribute to cancer susceptibility and concurs with the enhanced skin carcinogenesis seen in MMP-1 transgenic mice [39].

MMPs as negative regulators of neoplastic growth: release of bioactive ECM fragments

Historically, MMPs were thought to facilitate neoplastic progression by merely degrading ECM structural components, thereby allowing a cleared path for migrating tumor cells. It is now clear, however, that cryptic protein fragments exist within some ECM proteins that are released by proteolytic cleavage and which affect critical parameters of tumor evolution (e.g. angiogenesis and metastasis [17]). The first example for the release of a bioactive ECM fragment was the isolation of angiostatin from the urine of mice with Lewis lung cell carcinoma [40]. Angiostatin, a plasminogen cleavage product containing kringle regions 1–4, inhibits endothelial cell proliferation, is believed

responsible for maintaining Lewis lung cell metastases in a dormant state [40], and has shown efficacy in limiting tumor burden in transgenic mice predisposed to *de novo* development of pancreatic islet cell carcinomas [41••].

One of the first enzymes identified that was capable of generating angiostatin from plasminogen was macrophage-derived metalloelastase/MMP-12 [42]. Subsequently, three additional MMPs have been demonstrated to possess angiostatin-converting enzyme capabilities (e.g. matrilysin/MMP-7, gelatinase B/MMP-9, and gelatinase A/MMP-2 [43,44]). In addition to angiostatin, other proteolytic fragments — most prominent being endostatin, a 20 kDa carboxy-terminal fragment of type XVIII collagen — effectively inhibits tumor angiogenesis [45]. Endostatin, like angiostatin, has generated significant interest as it also limits tumor burden in transgenic mice in a stage-specific manner during neoplastic development [41••].

Further evidence to support the role of pericellular enzymes in regulating angiogenesis comes from this emerging theme that potent angiogenesis inhibitors are embedded fragments of larger proteins with disparate functions [17,46]. As tumors acquire *locally* invasive characteristics, perhaps as a function of increased MMP expression, metastatic capability may be suppressed during early neoplastic growth. Furthermore, increased permeability of the tumor vasculature may sequester circulating plasminogen within the tumor stroma where plasminogen is cleaved into angiostatin that then, in turn, suppresses metastatic spread [44]. Thus with respect to the chemotherapeutic use of MMP inhibitors, it will be important to understand the precise role MMPs play in modulating angiogenesis as well as their role during later neoplastic events.

Conclusions

Collectively, the body of data at present indicates that proteinases may benefit either the host or the tumor depending upon tumor stage, spatial expression, proteolytic capacity and binding affinity for matrix versus neoplastic cells. Nevertheless, variably specific MMP inhibitors are effective in inhibiting growth of various primary tumors and metastases in animal models [41••,46]. The ability of these compounds to inhibit tumor growth suggests that metalloproteinases are more important for tumor development than for normal physiological activities in the adult host. Thus, it has been postulated that development of more specific proteinase inhibitors may represent tractable chemotherapeutics for human neoplasia [46]. As a result many pharmaceutical companies have designed novel drugs that variably block MMP activity.

Notably, however, the Bayer Corporation recently announced that it was halting their clinical trial of an MMP-2 and MMP-9 inhibitor (BAY 12-9566) as "patients who took the drug experienced greater progression of cancer than the half who took placebo" [47]. These results are somewhat surprising and contrary to Bayers' preclinical data, which confirmed that the drug inhibited tumor activity in rodents. A body of data over the past few years indicate, however, that proteinases and proteinase inhibitors may, under specific circumstance, either favor or block tumor progression. For example, ectopic expression of TIMP-1 allows some tumors to grow, while inhibiting others [12••,37••,48]. In addition, gelatinase B/MMP-9-deficient transgenic mice predisposed to *de novo* squamous carcinomas [49], although developing 30% fewer tumors compared to controls, consistently elaborate squamous carcinomas of a higher malignant grade (LM Coussens, D Hanahan, Z Werb, unpublished data).

Although the mechanism(s) responsible for these results are not clear, the data argue that perhaps the most efficacious window for inhibitor-based therapy may be during early neoplastic progression (onset of angiogenesis and carcinoma *in situ*) prior to malignant conversion and/or frank tumor growth. Alternatively, combined administration of MMP inhibitors with anti-angiogenic agents, such as angiostatin or endostatin, or with immuno-therapeutics, may prove optimal in clinical treatment of particular tumors. Regardless, it is clear that metalloendoproteinases regulate many critical events in addition to tumor cell invasion (e.g. liberation of bioactive fragments of ECM, release of sequestered growth factors and angiogenic mediators) and in the shedding of cell surface receptors and ligands effecting bioavailability of chemokines and cytokines (Figure 4), all of which represent critical extrinsic events that variably regulate neoplastic cell proliferation, tumor-associated angiogenesis and inflammation, tumor growth, and metastasis formation. Further elucidation of the pathways downstream of proteolytic activity will be critical for defining new molecular targets. Thus, the behavior of neoplastic cells can no longer be understood without taking into account their active and reciprocal dialogue with the dynamic microenvironment in which they live.

Acknowledgements

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References and recommended reading

Papers of particular interest, published within the annual period of review, have been highlighted as:

- • of special interest
- •• of outstanding interest

1. Coussens LM, Werb Z: **Matrix metalloproteinases and the development of cancer.** *Chem Biol* 1996, 3:895-904.

2. Werb Z: **ECM and cell surface proteolysis: regulating cellular ecology.** *Cell* 1997, 91:439-442.

3. • Lukashev ME, Werb Z: **ECM signaling: orchestrating cell behavior and misbehavior.** *Trends Cell Biol* 1998, 8:437-441.
This paper, together with [2], is an outstanding review describing new targets of proteinase action.

4. Rawlings ND, Barrett AJ: **Evolutionary families of metallopeptidases.** *Methods Enzymol* 1995, 248:183-228.

5. Stöcker W, Grams F, Baumann U, Reinemer P, Gomis-Rüth FX, McKay DB, Bode W: **The metzincins – topological and sequential relations between the astacins, adamalysins, serralysins, and matrixins (collagenases) define a superfamily of zinc-peptidases.** *Protein Sci* 1995, 4:823-840.

6. Wolfsberg TG, White JM: **ADAMs in fertilization and development.** *Dev Biol* 1996, 180:389-401.

7. Black RA, White JM: **ADAMs: focus on the protease domain.** *Curr Opin Cell Biol* 1998, 10:654-659.

8. •• Vázquez F, Hastings G, Ortega M-A, Lane TF, Oikemus S, Lombardo M, Iruela-Arispe ML: **METH-1, a human ortholog of ADAMTS-1, and METH-2 are members of a new family of proteins with angio-inhibitory activity.** *J Biol Chem* 1999, 274:23349-23357.

See annotation [10••].

9. •• Hurskainen TL, Hirohata S, Seldin MF, Apte SS: **ADAM-TS5, ADAM TS6, and ADAM-TS7, novel members of a new family of zinc metalloproteases. General features and genomic distribution of the ADAM-TS family.** *J Biol Chem* 1999, 274:25555-25563.

See annotation [10••].

10. •• Tang BL, Hong W: **ADAMTS: a novel family of proteases with an ADAM protease domain and thrombospondin 1 repeats.** *FEBS Lett* 1999, 445:223-225.

A short review describing and characterizing the structural homology of four ADAMTS and three adamylisin-related proteinases.

11. Naïk P, Christofori G, Hanahan D: **Insulin-like growth factor II is focally upregulated and functionally involved as a second signal for oncogene-induced tumorigenesis.** *Cold Spring Harbor Symp Quant Biol* 1994, 59:459-470.

12. •• Martin DC, Fowlkes JL, Babic B, Khokha R: **Insulin-like growth factor II signaling in neoplastic proliferation is blocked by transgenic expression of the metalloproteinase inhibitor TIMP-1.** *J Cell Biol* 1999, 146:881-892.

An elegant *in vivo* study showing that regulation of hepatic tumor growth by IGF II is dependent on the balance of MMPs and their TIMP inhibitors. Degradation of IGF-binding proteins results in bioavailability of IGF II and IGF-I receptor signaling that is blocked by TIMP-1. These data indicate that IGF bioavailability is subject to control by MMP-mediated degradation of IGF-binding proteins.

13. Oh Y: **IGFBPs and neoplastic models. New concepts for roles of IGFBPs in regulation of cancer cell growth.** *Endocrine* 1997, 7:111-113.

14. Fowlkes JL, Enghild JJ, Suzuki K, Nagase H: **Matrix metalloproteinases degrade insulin-like growth factor-binding protein-3 in dermal fibroblast cultures.** *J Biol Chem* 1994, 269:25742-25746.

15. Mañes S, Mira E, del Mar Barbacid M, Ciprés A, Fernández-Resa P, Maria Buesa J, Mérida I, Aracil M, Márquez G, Martínez-AC: **Identification of insulin-like growth factor-binding protein-1 as a potential physiological substrate for human stromelysin-3.** *J Biol Chem* 1997, 272:25706-25712.

16. Mañes S, Llorente M, Lacalle RA, Gómez-Moutón C, Kremer L, Mira E, Martínez-A C: **The matrix metalloproteinase-9 regulates the insulin-like growth factor-triggered autocrine response in DU-145 carcinoma cells.** *J Biol Chem* 1999, 274:6935-6945.

17. Hanahan D, Folkman J: **Patterns and emerging mechanisms of the angiogenic switch during tumorigenesis.** *Cell* 1996, 86:353-364.

18. Whitelock JM, Murdoch AD, Iozzo RV, Underwood AP: **The degradation of human endothelial cell-derived perlecan and release of bound basic fibroblast growth factor by stromelysin, collagenase, plasmin, and heparanases.** *J Biol Chem* 1996, 271:10079-10086.

19. Czubayko F, Liaudet-Coopman EDE, Aigner A, Tuveson AT, Berchem GJ, Wellstein A: **A secreted FGF-binding protein can serve as the angiogenic switch in human cancer.** *Nature* 1997, 3:1137-1140.

20. Arribas J, Coodly L, Vollmer P, Kishimoto TK, Rose-John S, Massagué J: **Diverse cell surface protein ectodomains are shed by a system sensitive to metalloprotease inhibitors.** *J Biol Chem* 1996, 271:11376-11382.

21. Tracey K, Cerami A: **Tumor necrosis factor, other cytokines and disease.** *Annu Rev Cell Biol* 1993, 9:317-443.

22. •• Coussens LM, Raymond WW, Bergers G, Laig-Webster M, Behrendtsen O, Werb Z, Caughey GH, Hanahan D: **Inflammatory mast cells up-regulate angiogenesis during squamous epithelial carcinogenesis.** *Genes Dev* 1999, 13:1382-1397.

The authors demonstrate in a transgenic mouse model of squamous cell carcinogenesis that angiogenesis is regulated in a biphasic stage-specific manner by inflammatory host cells and tumor cells. In the first phase, mast cells potentiate an early angiogenic response in hyperplastic/dysplastic tissue by expressing enzymes that are implicated in stromal remodeling and neovascularization. At the tumor stage, cancers are deprived of mast cells but the tumor cells themselves sustain angiogenesis by upregulating angiogenesis factors.

23. Black RA, Rauch CT, Kozlosky CJ, Peschon JJ, Slack JL, Wolfson MF, Castner BJ, Stocking KL, Reddy P, Srinivasan S *et al.*: **A metalloproteinase disintegrin that releases tumor-necrosis factor-α cells.** *Nature* 1997, 385:729-733.

24. Moss ML, Jin S-LC, Milla ME, Burkhart W, Carter HL, Chen W-J, Clay WC, Didsbury JR, Hassler D, Hoffman CR *et al.*: **Cloning of a disintegrin metalloproteinase that processes precursor tumor-necrosis factor-α.** *Nature* 1997, 385:733-736.

25. •• Peschon JJ, Slack JL, Reddy P, Stocking KL, Sunnarborg SW, Lee DC, Russell WE, Castner BJ, Johnson RS, Fitzner JN *et al.*: **An essential role for ectodomain shedding in mammalian development.** *Science* 1998, 282:281-284.

Analysis of *ADAM17/TACE* gene knockout mice reveals an extended substrate repertoire for the enzyme, implicating ectodomain shedding by metalloproteinases as a general mechanism regulating autocrine and paracrine signaling of several membrane-bound growth factors.

26. •• Moore RJ, Owens DM, Stamp G, Arnott C, Burke F, East N, Holdsworth H, Turner L, Rollins B, Pasparakis M *et al.*: **Mice deficient in tumor necrosis factor-α are resistant to skin carcinogenesis.** *Nature* 1999, 5:828-831.

An excellent study describing the necessity of TNF-α as a pro-inflammatory factor in the two-step skin carcinogenesis model. Utilizing TNF-α deficient mice, the authors demonstrate that TNF-α is required for *de novo* carcinogenesis at early stages of tumor promotion. This study and the one described [22••] support the notion that host inflammatory components play a significant role in early skin carcinogenesis.

27. Dong J, Opresko LK, Dempsey PJ, Lauffenburger DA, Coffey RJ, Wiley HS: **Metalloprotease-mediated ligand release regulates autocrine signaling through the epidermal growth factor receptor.** *Proc Natl Acad Sci USA* 1999, 96:6235-6240.

28. Suzuki M, Raab G, Moses MA, Fernandez CA, Klagsbrun M: **Matrix metalloproteinase-3 releases active heparin-binding EGF-like growth factor by cleavage at a specific juxtamembrane site.** *J Biol Chem* 1997, 272:31730-31737.

29. Gallea-Robache S, Morand V, Millet S, Bruneau JM, Bhatnagar N, Chouaib S, Roman-Roman S: **A metalloproteinase inhibitor blocks the shedding of soluble cytokine receptors and processing of transmembrane cytokine precursors in human monocytic cells.** *Cytokine* 1997, 9:340-346.

30. Amour A, Slocombe PM, Webster A, Butler M, Knight CG, Smith BJ, Stephens PE, Shelley C, Hutton M, Knauper V *et al.*: **TNF-alpha converting enzyme (TACE) is inhibited by TIMP-3.** *FEBS Lett* 1998, 435:39-44.

31. Lombard MA, Wallace TL, Kubicek MF, Petzold GL, Mitchell MA, Hendges SK, Wilks JW: **Synthetic matrix metalloproteinase inhibitors and tissue inhibitor of metalloproteinase (TIMP)-2, but not TIMP-1, inhibit shedding of tumor necrosis factor-alpha receptors in a human colon adenocarcinoma (Colo 205) cell line.** *Cancer Res* 1998, 58:4001-4017.

32. •• Christofori G, Semb H: **The role of the cell-adhesion molecule E cadherin as a tumor-suppressor gene.** *Trends Biochem Sci* 1999, 24:73-76.

A comprehensive review describing that loss of E-Cadherin during the development of most epithelial cancers may not only contribute to loss of cell–cell adhesion, but also convey signals that actively induce tumor cell invasion and metastasis, thus, implicating E-Cadherin as a potential tumor suppressor gene.

33. •• Perl A-K, Wilgenbus P, Dahl U, Semb H, Christofori G: **A causal role for E-cadherin in the transition from adenoma to carcinoma.** *Nature* 1998, 392:190-193.

An elegant study investigating the functional importance of E-Cadherin in a transgenic mouse model of islet carcinogenesis. Re-establishment of intact E-Cadherin complexes results in arrest of tumor development at the benign adenoma stage, whereas expression of a dominant-negative form of E-cadherin induces early invasion and metastasis. These results demonstrate that loss of E-cadherin-mediated cell adhesion is one rate-limiting step in the progression from benign adenomas to malignant carcinoma.

34. Lochter A, Galosy S, Muschler J, Freedman N, Werb Z, Bissell MJ: **Matrix metalloproteinase stromelysin-1 triggers a cascade of molecular alterations that leads to stable epithelial-to-mesenchymal conversion and a premalignant phenotype in mammary epithelial cells.** *J Cell Biol* 1997, 139:1861-1872.

35. Sternlicht MD, Bissell MJ, Werb Z: **The matrix metalloproteinase stromelysin-1 acts as a natural mammary tumor promoter.** *Oncogene* 2000, in press.

36. Lochter A, Srebrow A, Sympson CJ, Terracio N, Werb Z, Bissell MJ: **Misregulation of stromelysin-1 expression in mouse mammary tumor cells accompanies acquisition of stromelysin-1-dependent invasive properties.** *J Biol Chem* 1997, 272:5007-5015.

37. •• Sternlicht MD, Lochter A, Sympson CJ, Huey B, Rougier J-P, Gray JW, Pinkel D, Bissell MJ, Werb Z: **The stromal proteinase MMP3/stromelysin-1 promotes mammary carcinogenesis.** *Cell* 1999, 98:137-146.

The first *in vivo* demonstration where over-expression of an extracellular proteinase, stromelysin-1, in the stromal compartment of mammary glands is able to promote spontaneous premalignant changes and malignant conversion to mesenchymal-like tumors. Once inititiated, tumors become independent of stromelysin-1 and show specific genomic changes. These data indicate that stromelysin-1 influences tumor initiation and alters neoplastic risk.

38. • Rutter JL, Mitchell TI, Buttice G, Meyers J, Gusella JF, Ozelius LJ, Brinckerhoff CE: **A single nucleotide polymorphism in the matrix metalloproteinase-1 promoter creates an Ets binding site and augments transcription.** *Cancer Res* 1998, 58:5321-5325.

This work raises the issue that the search for cancer susceptibility loci – containing mutations, amplifications, and/or polymorphisms – should expand to include genes encoding extracellular proteinases.

39. D'Armiento J, DiColandrea T, Dalal SS, Okada T, Huang MT, Conney AH, Chada K: **Collagenase expression in transgenic mouse skin causes hyperkeratosis and acanthosis and increases susceptibility to tumorigenesis.** *Mol Cell Biol* 1995, 15:5732-5739.

40. O'Reilly MS, Holmgren L, Shing Y, Chen C, Rosenthal RA, Moses M, Lane WS, Cao Y, Sage EH, Folkman J: **A novel angiogenesis inhibitor which mediates the suppression of metastasis by a Lewis Lung carcinoma.** *Cell* 1994, 79:315-328.

41. •• Bergers G, Javaherian K, Lo KM, Folkman J, Hanahan D: **Effects of angiogenesis inhibitors on multistage carcinogenesis in mice.** *Science* 1999, 284:808-812.

Using transgenic mice, predisposed to *de novo* islet cell carcinogenesis, as a preclinical testing model, the authors have examined stage-specific efficacy of several potent angiogenesis- and MMP-inhibitors on neoplastic progression. The work clearly demonstrates differential efficacy of tested therapeutics dependent upon neoplastic stage.

42. Dong Z, Kumar R, Yang X, Fidler IJ: **Macrophage-derived metalloelastase is responsible for the generation of angiostatin in lewis lung carcinoma.** *Cell* 1997, 88:801-810.

43. Patterson, BC Xiang Q, Sang A: **Angiostatin-converting enzyme activities of human matrilysin (MMP-7) and gelatinase B/type IV collagenase (MMP-9).** *J Biol Chem* 1997, 272:28823-28825.

44. O'Reilly MS, Wiederschain D, Stetler-Stevenson WG, Folkman J, Moses MA: **Regulation of angiostatin production by matrix metalloproteinase-2 in a model of concomitant resistance.** *J Biol Chem* 1999, 274:29568-29571.

45. O'Reilly M, Boehm T, Shing Y, Fukai N, Vasios G, Lane WS, Flynn E, Birkhead JR, Olsen BR, Folkman J: **Endostatin: an endogenous inhibitor of angiogenesis and tumor growth.** *Cell* 1997, 88:277-285.

46. Brown PD: **Matrix metalloproteinase inhibitors in the treatment of cancer.** *Med Oncol* 1997, 14:1-10.

47. Pierson R: **Bayer ends trial of novel cancer, arthritis drug.** http://news.lycos.com/stories/Science/19990926RTSCIENCE-HEALTH-BAYER.asp

48. Soloway P, Alexander CM, Werb Z, Jaenisch R: **Targeted mutagenesis of TIMP-1 reveals that experimental metastasis is influenced by the TIMP-1 genotype of the tumor but not by that of the host.** *Oncogene* 1996. 13:2307-2314.

49. Coussens LM, Hanahan D, Arbeit JM: **Genetic predisposition and parameters of malignant progression in K14-HPV16 transgenic mice.** *Am J Pathol* 1996, 149:1899-1917.